

Efficacy of Vitamin K₂ for Glucocorticoid-induced Osteoporosis in Patients with Systemic Autoimmune Diseases

Kotaro Shikano¹, Kaichi Kaneko¹, Mai Kawazoe¹, Makoto Kaburaki¹,
Tomoko Hasunuma^{1,2} and Shinichi Kawai¹

Abstract

Objective Vitamin K₂ (menatrenone) is an effective treatment for patients with postmenopausal osteoporosis. We herein performed a subanalysis of patients with systemic autoimmune diseases undergoing glucocorticoid therapy in our previous prospective study.

Methods Sixty patients were categorized into a group with vitamin K₂ treatment (n=20, Group A) and a group without vitamin K₂ treatment (n=40, Group B). All patients were treated with bisphosphonates.

Results Serum levels of osteocalcin and undercarboxylated osteocalcin decreased significantly after the start of glucocorticoid therapy in both groups, while the serum osteocalcin level was significantly higher in Group A than Group B during the third (p=0.0250) and fourth weeks (p=0.0155). The serum level of the N-terminal peptide of type I procollagen, a bone formation marker, decreased during glucocorticoid therapy, but was significantly higher in Group A than Group B during the fourth week (p=0.0400). The bone mineral density and fracture rate showed no significant differences between the two groups.

Conclusion Although vitamin K₂ improves bone turnover markers in patients with osteoporosis on glucocorticoid therapy, it has no significant effect on the bone mineral density and fracture rate after 1.5 years of treatment.

Key words: vitamin K₂ (menatrenone), glucocorticoid, osteoporosis, osteocalcin

(Intern Med 55: 1997-2003, 2016)

(DOI: 10.2169/internalmedicine.55.6230)

Introduction

Glucocorticoids are widely used to treat various diseases, including systemic autoimmune diseases. Although glucocorticoid therapy can improve the outcome of patients with these diseases, various side effects of long-term treatment have become major problems. Osteoporosis is particularly serious because it can impair daily activities by leading to critical bone injuries such as vertebral or femoral neck fractures (1-4). Bisphosphonates were recommended as first-line therapy in a recently revised guideline on the management and treatment of glucocorticoid-induced osteoporosis published by the Japanese Society for Bone and Mineral Research (5).

Osteocalcin (OC) is produced by osteoblasts and involved

in calcification of the bone. Inside the osteoblasts, OC is metabolized to γ -carboxylated OC (GlaOC) by γ -glutamyl carboxylase. GlaOC has a high affinity for hydroxyapatite in bone tissue and it combines with calcium in hydroxyapatite to contribute to the formation of the three-dimensional architecture of the bone. Vitamin K₂ (menatrenone) is required for γ -carboxylation of glutamate residues on OC. If vitamin K₂ is lacking, then GlaOC concentrations decrease and osteoblasts release undercarboxylated OC (ucOC) into the blood (6, 7). It has been reported that a high serum level of ucOC, which suggests decreased production of GlaOC, is positively correlated with a high incidence of fracture (8). It has also been reported that the serum ucOC level is negatively correlated with the bone mineral density (BMD) (9, 10). Several studies have suggested that vitamin K₂ increases the BMD in postmenopausal osteoporosis pa-

¹Division of Rheumatology, Department of Internal Medicine, School of Medicine, Faculty of Medicine, Toho University, Japan and ²Clinical Pharmacology Center, Oita University Faculty of Medicine, Japan

Received for publication July 23, 2015; Accepted for publication November 3, 2015

Correspondence to Dr. Kaichi Kaneko, ihciakokenak@med.toho-u.ac.jp

tients (11-16). However, there is little clinical evidence regarding the efficacy of vitamin K₂ for glucocorticoid-induced osteoporosis. We previously performed a prospective study that assessed changes in bone turnover markers during glucocorticoid therapy (17). Using the database from this study, the patients using glucocorticoids were categorized into groups with and without concomitant vitamin K₂ therapy. We then performed a post-hoc subanalysis that examined the efficacy of vitamin K₂ for glucocorticoid-induced osteoporosis.

Materials and Methods

Patients and study design

All patients were recruited at Toho University Omori Medical Center. This was a prospective observational study that enrolled 60 patients with systemic autoimmune diseases, including 21 patients with systemic lupus erythematosus (SLE), 15 patients with polymyositis/dermatomyositis (PM/DM), 19 patients with vasculitis syndrome, and five patients with adult-onset Still's disease. All patients who commenced treatment with prednisolone at doses from 30 to 60 mg daily [mean daily dose: 45.2±1.9 mg (SEM)] according to our standard regimen were eligible for this study. All patients provided their written informed consent before enrollment and this study was approved by the Ethics Committee of Toho University Omori Medical Center (approval no. 21-61 and 24-78). None of the subjects had received any treatment for their diseases at the time of enrollment.

Concomitant administration of a bisphosphonate (alendronate 35 mg/week or risedronate 17.5 mg/week) was performed with high-dose glucocorticoid therapy in all patients, but none of the patients received calcium or vitamin D supplements during this study. Twenty patients received vitamin K₂ at 45 mg daily from the start of glucocorticoid therapy, while the other 40 patients did not. The vitamin K₂ dosage was not changed during the study period. Whether or not the patient received vitamin K₂ therapy was determined by the attending physician.

Fasting morning blood samples were collected just before the patients started treatment and after 1, 2, 3, and 4 weeks of glucocorticoid therapy. Serum samples were collected and immediately frozen at -80°C until the measurement of bone metabolism markers.

Serum parameters

The serum levels of OC (Mitsubishi Kagaku Bioclinical Laboratories, Tokyo, Japan) and N-terminal peptide of type I procollagen (PINP; Orion Diagnostica, Espoo, Finland) were determined by an immunoradiometric assay. The serum level of ucOC (Sanko Junyaku, Tokyo, Japan) was measured by an electrochemiluminescence immunoassay, while bone alkaline phosphatase (BAP; Quidel, San Diego, USA) was measured by an enzyme immunoassay. The serum levels of cross-linked N-telopeptide of type I collagen (NTX; Inver-

ness, Princeton, USA) and tartrate-resistant acid phosphatase isoform 5b (TRACP-5b; DS Pharma Biomedical, Tokyo, Japan) were determined by enzyme immunoassays.

Measurement of the bone mineral density (BMD)

Before starting glucocorticoid therapy, the BMD of the lumbar spine (L2-4) was measured by dual-energy X-ray absorptiometry using Discovery A (Hologic, Waltham, USA). The BMD was automatically calculated from the bone area (cm²) and bone mineral content (g) and expressed in g/cm². Measurement of the BMD was repeated after 15±4 months of glucocorticoid therapy and the percent change from baseline was calculated.

Statistical analysis

Statistical analysis was performed with the StatFlex software program (ver.6; ARTEC, Osaka, Japan). Numerical data are expressed as the mean±SEM and as the median with the interquartile range. Assessment of the changes during glucocorticoid therapy was performed by Dunnett's multiple comparison test with Bonferroni's correction. When comparing two groups, the Mann-Whitney *U* test was applied for numerical data and Fisher's exact test was used for categorical data. The log-rank test was employed to assess the significance of differences in fracture rates. *p* values of <0.05 were considered to be statistically significant.

Results

Clinical profile

As shown in Table, 60 patients were categorized into a group receiving vitamin K₂ (n=20, Group A) and a group without vitamin K₂ supplementation (n=40, Group B). There were no significant differences between these two groups with respect to age, sex, or menopausal status. The BMI was significantly larger in Group B. The percentages of patients with SLE, PM/DM, vasculitis syndrome, or adult onset Still's disease were not significantly different between the two groups. There were no significant between-group differences in the baseline serum levels of OC, ucOC, PINP, BAP, or NTX. Moreover, the mean initial and cumulative doses of prednisolone did not differ between Groups A and B or between premenopausal and postmenopausal patients.

Bone turnover markers

Fig. 1 shows the serum level of OC at baseline and during glucocorticoid therapy. The OC levels decreased significantly during the first week of glucocorticoid therapy in both groups and remained low at 4 weeks in Group B. The serum OC level [mean±SEM] was significantly higher in Group A than Group B during the third week (0.985±0.138 vs. 0.518±0.083 ng/mL; *p*=0.025) and the fourth week of glucocorticoid therapy (1.070±0.172 vs. 0.470±0.078 ng/mL; *p*=0.0155), respectively.

Fig. 2 displays the serum levels of ucOC [mean±SEM]

Table. Baseline Demographic and Clinical Data of the Groups with and without Vitamin K₂ (Menatetrenone) Supplementation.

	Vitamin K ₂		p value
	with (n=20, Group A)	without (n=40, Group B)	
Age (yr)	55.7±4.6	55.0±2.5	p=0.869 ^a
Number of men/women	9/11	12/28	p=0.563 ^b
Postmenopausal women (%)	6 (54.5)	17 (60.7)	p=0.728 ^b
BMI (kg/m ²)	19.9±0.7	22.6±0.72	p=0.038 ^{a*}
BMD (g/cm ²)	0.938±0.034	0.938±0.024	p=0.927 ^a
Systemic autoimmune disease			
SLE (n=21)	5 (25%)	16 (40%)	p=0.389 ^b
PM/DM (n=15)	5 (25%)	10 (25%)	p=1.000 ^b
Vasculitis syndrome (n=19)	9 (45%)	10 (25%)	p=0.146 ^b
AOSD (n=5)	1 (5%)	4 (10%)	p=0.656 ^b
Serum markers:			
OC (ng/mL)	2.64±0.32 2.35 [1.45-3.50]	2.21±0.24 2.00 [1.00-3.30]	p=0.286 ^a
ucOC (ng/mL)	1.66±0.40 1.05 [0.45-2.25]	1.51±0.24 0.91 [0.61-2.40]	p=0.925 ^a
PINP (μg/L)	48.5±8.2 35.9 [29.0-58.4]	42.5±3.0 39.1 [26.9-50.8]	p=0.845 ^a
BAP (U/L)	16.9±1.4 13.8 [12.4-21.6]	15.5±1.0 14.4 [11.3-18.5]	p=0.525 ^a
NTX (nmol BCE/L)	16.6±1.2 15.7 [13.6-19.6]	14.3±0.6 14.0 [11.8-16.4]	p=0.063 ^a
Initial glucocorticoid dose /BW (mg/kg)	0.786±0.053	0.861±0.034	p=0.093 ^a
Total glucocorticoid dose (mg)	9159±759	9453±364	p=0.262 ^a

Data are shown as the mean ± SEM and as the median [25th to 75th percentiles]. * p<0.05 between patients with and without vitamin K₂; ^a Mann-Whitney *U* test. ^b Fisher's exact test. BMI: body mass index, BMD: bone mineral density, SLE: systemic lupus erythematosus, PM/DM: polymyositis/dermatomyositis, AOSD: adult-onset Still's disease, OC: osteocalcin, ucOC: undercarboxylated osteocalcin, PINP: N-terminal peptide of type I procollagen, BAP: bone alkaline phosphatase, NTX: cross-linked N-telopeptide of type I collagen, BW: body weight

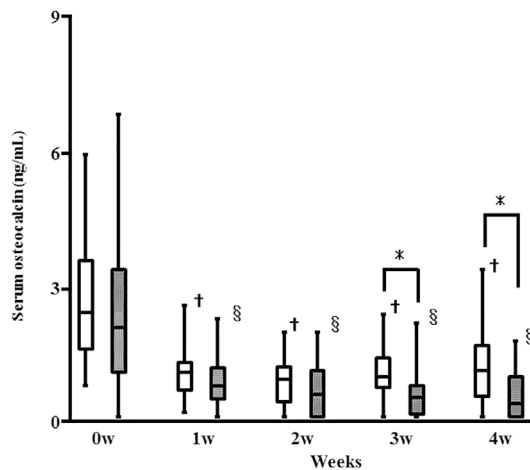


Figure 1. Serum levels of OC in patients with (Group A) or without (Group B) vitamin K₂ supplementation during glucocorticoid therapy. Data are expressed as the median with 25th to 75th percentiles. Open columns indicate Group A and shaded columns indicate Group B. †, §; p<0.01 vs. baseline by Dunnett's multiple comparison test (with Bonferroni's correction). *; p<0.05 between groups by the Mann-Whitney *U* test.

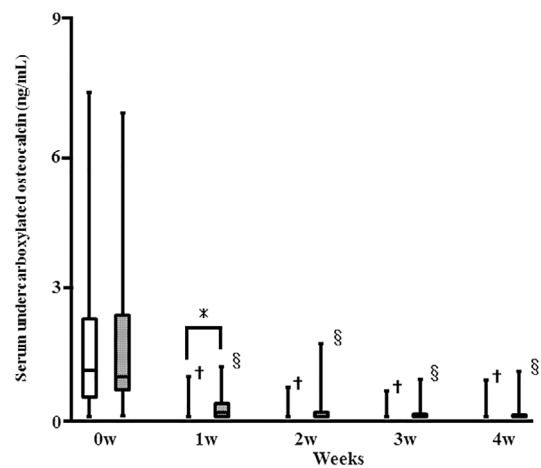


Figure 2. Serum levels of ucOC in patients with (Group A) or without (Group B) vitamin K₂ supplementation during glucocorticoid therapy. Data are expressed as the median with 25th to 75th percentiles. Open columns indicate Group A and shaded columns indicate Group B. †, §; p<0.01 vs. baseline by Dunnett's multiple comparison test (with Bonferroni's correction). *; p<0.05 between groups by the Mann-Whitney *U* test.

at baseline and during glucocorticoid therapy. The serum ucOC levels showed a significant decrease during the first week of glucocorticoid therapy in both groups and remained

reduced during the fourth week in Group B. Only during the first week of therapy was the serum level of ucOC significantly lower in Group A than Group B (0.118 ± 0.065 vs.

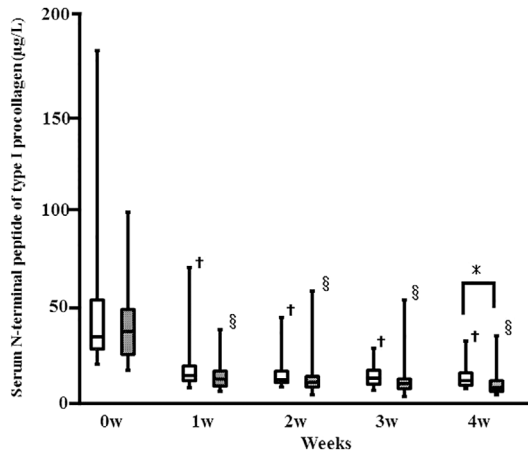


Figure 3. Serum levels of PINP in patients with (Group A) or without (Group B) vitamin K₂ supplementation during glucocorticoid therapy. Data are expressed as the median with 25th to 75th percentiles. Open columns indicate Group A and shaded columns indicate Group B. †, §; p<0.01 vs. baseline by Dunnett's multiple comparison test (with Bonferroni's correction). *; p<0.05 between groups by the Mann-Whitney U test.

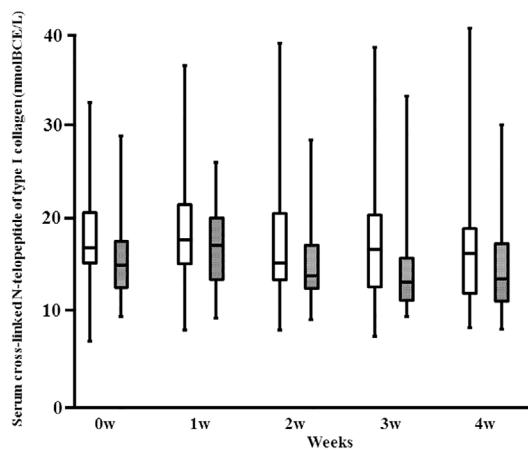


Figure 5. Serum levels of NTX in patients with (Group A) or without (Group B) vitamin K₂ supplementation during glucocorticoid therapy. Data are expressed as the median with 25th to 75th percentiles. Open columns indicate Group A and shaded columns indicate Group B.

0.214 ± 0.045 ng/mL; p=0.0326).

Fig. 3 shows the changes in the serum level of PINP [mean ± SEM], a bone formation marker, during glucocorticoid therapy. Serum PINP levels continued to decrease from the first week to the fourth week of therapy in both groups. During the fourth week, the serum PINP level was significantly higher in Group A than Group B (14.780 ± 1.443 vs. 10.988 ± 0.858 µg/L; p=0.0400).

Figs. 4 and 5 display the serum levels of BAP and NTX, respectively. These markers did not change markedly during glucocorticoid therapy in either group.

BMD

Fig. 6 reveals the changes in the BMD [mean ± SEM]

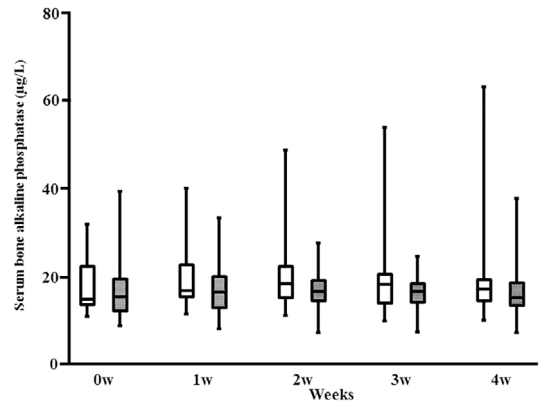


Figure 4. Serum levels of BAP in patients with (Group A) or without (Group B) vitamin K₂ supplementation during glucocorticoid therapy. Data are expressed as the median with 25th to 75th percentiles. Open columns indicate Group A and shaded columns indicate Group B.

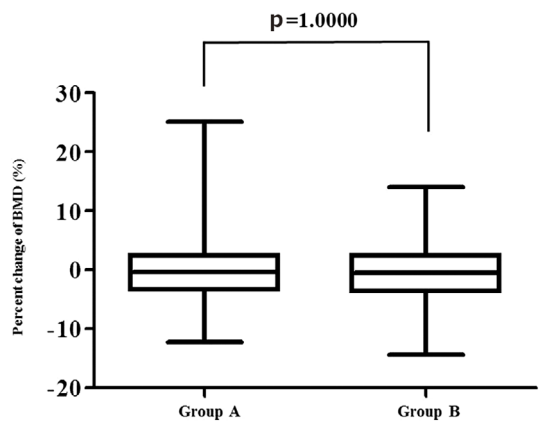


Figure 6. Changes in the BMD during glucocorticoid therapy in patients with (Group A) or without (Group B) vitamin K₂ supplementation. Data are expressed as the median with 25th to 75th percentiles and were analyzed by the Mann-Whitney U test.

during glucocorticoid therapy (mean period: 1.5 years). There was no significant difference between the two groups with regard to the changes in the BMD (Group A: -0.429 ± 1.077 vs. Group B: 0.507 ± 2.396%; p=1.0000).

Fracture

The rate of new fractures was 0% (0/20 patients) [0/30.2=0% per patient-year] in Group A and 5% (2/40 patients) [2/59.8=3.3% per patient-year] in Group B during glucocorticoid therapy (mean duration: 1.5 years), showing no significant difference between the two groups (p=0.5480). Fig. 7 displays a comparison of the fracture rates by the Kaplan-Meier method, which also revealed no significant difference between the two groups in the percentage of patients without fracture (p=0.3013).

Two patients had fractures in Group B, and both were postmenopausal women with vasculitis syndrome. One patient was an 84-year-old woman taking prednisolone (30

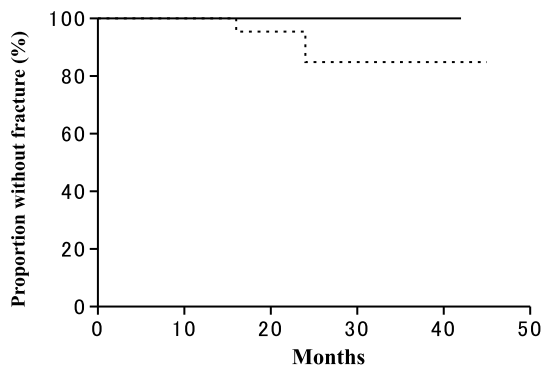


Figure 7. A Kaplan-Meier analysis of the percentage of patients without fracture during glucocorticoid therapy in Group A and Group B (with or without vitamin K₂ supplementation, respectively). Group A, unbroken line; Group B, broken line.

mg/day) and alendronate (35 mg/week) who developed a lumbar compression fracture after 15 months of glucocorticoid therapy. The other patient was a 65-year-old woman using prednisolone (50 mg/day) and alendronate (35 mg/week) who developed a left rib fracture after 24 months of glucocorticoid therapy.

Discussion

The present study showed that the serum levels of OC and ucOC were decreased after glucocorticoid therapy. D'vivo et al. (18) investigated 13 patients with multiple sclerosis receiving glucocorticoid therapy for 10 days and detected a significant decrease in the serum OC level from day 1. Kuroki et al. (19) found that the serum OC levels were significantly decreased by glucocorticoid therapy in 47 patients with collagen, hematological, and neuroimmune diseases. Kaji et al. (20) reported that the serum level of OC was significantly decreased by glucocorticoid therapy in patients with collagen, hematopoietic, and neuroimmune diseases. In addition, Kauh et al. (21) reported that the serum OC level was significantly decreased by glucocorticoid administration in healthy adults. While our findings were similar to these reported results, the serum level of ucOC was not measured in the previous studies. We also compared changes in the serum levels of bone turnover markers during glucocorticoid therapy between patients with and without vitamin K₂ supplementation. We found that the serum levels of OC and ucOC showed significant improvement with the administration of vitamin K₂, but the BMD and the incidence of fracture were not altered.

It has been reported that vitamin K₂ influences the bone metabolism by accelerating the transformation of OC to GlaOC (22) and increasing GlaOC concentrations promote calcification (23). It was also reported that vitamin K₂ promoted OC production by mouse bone marrow-derived mesenchymal stem cells (24). In addition, the differentiation of osteoblastic cells from precursor cells is induced by vitamin K₂ (23).

Many studies have demonstrated the efficacy of vitamin K₂ supplementation for postmenopausal osteoporosis. The serum OC level and BMD are significantly increased by the administration of vitamin K₂ (11-16), and a reduction of fracture risk was also observed in the vitamin K₂-treated groups of three studies (12, 13, 25). On the other hand, few studies have assessed the efficacy of vitamin K₂ for glucocorticoid-induced osteoporosis, apart from two investigations in patients with chronic glomerulonephritis (26, 27). Yonemura et al. (26) analyzed 20 patients aged 25-30 years with chronic glomerulonephritis who were taking glucocorticoids with or without vitamin K₂ supplementation. In the vitamin K₂ group, the lumbar BMD was maintained in the 10th week, while it showed a significant decrease in the group without vitamin K₂ supplementation. The serum OC level was significantly decreased in the group without vitamin K₂ supplementation, although there was no significant difference in the OC levels between the two groups. Sasaki et al. (27) also investigated the efficacy of vitamin K₂ supplementation in 20 patients aged 15-49 years receiving glucocorticoid therapy for chronic glomerulonephritis. In the group without vitamin K₂ supplementation, the lumbar BMD showed a significant decrease after 6 and 12 months of glucocorticoid therapy, while the reduction of the lumbar BMD was not significant in the group receiving vitamin K₂ supplementation. The serum OC level decreased significantly from the first month of glucocorticoid therapy in both groups.

The results of these two studies suggest that vitamin K₂ can prevent a glucocorticoid-induced decrement of the BMD in patients with chronic glomerulonephritis. However, bisphosphonates were not used in these studies, unlike our study. In the present study, the BMD did not decrease in the groups with or without vitamin K₂ supplementation during glucocorticoid therapy, suggesting that a decrease in the BMD was prevented by the administration of bisphosphonates to all patients.

Type I collagen is a major component of bone tissue. PINP is derived from cleaving the N-terminal of type I procollagen produced in osteoblasts, with the serum PINP level being influenced by the production of type I collagen and thus it is a useful marker of bone formation. Ichikawa et al. (28) reported that vitamin K₂ might promote the production of bone collagen by activating the steroid and xenobiotic receptor in osteoblastic cells, which suggests that an improvement in the PINP level in patients receiving vitamin K₂ might be a direct effect of the vitamin itself.

In our study, there was no significant change in the serum level of BAP after glucocorticoid therapy, and previous studies (18-20) have also found no significant change in the serum BAP level with glucocorticoid therapy. In fact, BAP may not be directly regulated by glucocorticoids because the glucocorticoid response element is not present in the promoter region of the BAP gene (29). In contrast, the expression of the OC and PINP genes is directly downregulated by glucocorticoids (30).

NTX also showed no change with glucocorticoid therapy. Since all of the patients in our study were taking a bisphosphonate, which strongly inhibits osteoclastogenesis, this may have led to the stable NTX concentration observed during glucocorticoid therapy.

The serum levels of OC, ucOC, and PINP were decreased by glucocorticoid therapy, but were significantly improved in the patients given vitamin K₂. Our results suggest that vitamin K₂ had some effect on these bone turnover markers, although it did not show a significant influence on the bone mineral density and fracture rate.

Conclusion

This study revealed that vitamin K₂ supplementation improved bone turnover markers in patients with osteoporosis due to glucocorticoid therapy, although its effects on the bone mineral density and fracture rate were not significant after 1.5 years of treatment.

Author's disclosure of potential Conflicts of Interest (COI).

Shinichi Kawai: Honoraria, Eisai; Research funding, Eisai.

Acknowledgement

We thank Professor Chiaki Nishimura for advice on the statistical analysis, and Sonoko Sakurai for her valuable secretarial assistance.

References

- Longo DL. Osteoporosis: glucocorticoid-induced osteoporosis. In: Harrison's Principles of Internal Medicine. 18th ed. Fauci A, Kasper D, Hauser S, Jameson J, Loscalzo J, Eds. McGraw-Hill Medical, New York, 2012: 3135.
- Buttgereit F, Straub RH, Wehling M, Burmester GR. Glucocorticoids in the treatment of rheumatic disease: an update on the mechanisms of action. *Arthritis Rheum* **50**: 3408-3417, 2004.
- den Uyl D, Bultink IE, Lems WF. Glucocorticoid-induced osteoporosis. *Clin Exp Rheumatol* **29**: S93-S98, 2011.
- Maricic M. Update on glucocorticoid-induced osteoporosis. *Rheum Dis Clin North Am* **37**: 415-431, 2011.
- Suzuki Y, Nawata H, Soen S, et al. Guidelines on the management and treatment of glucocorticoid-induced osteoporosis of the Japanese Society for Bone and Mineral Research: 2014 update. *J Bone Miner Metab* **32**: 337-350, 2014.
- Hoang QQ, Sicheri F, Howard AJ, Yang DS. Bone recognition mechanism of porcine osteocalcin from crystal structure. *Nature* **425**: 977-980, 2003.
- Hauschka PV, Lian JB, Cole DE, Gundberg CM. Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiol Rev* **69**: 990-1047, 1989.
- Vergnaud P, Garnero P, Meunier PJ, Bréart G, Kamihagi K, Delmas PD. Undercarboxylated osteocalcin measured with a specific immunoassay predicts hip fracture in elderly women: the EPIDOS Study. *J Clin Endocrinol Metab* **82**: 719-724, 1997.
- Szulec P, Arlot M, Chapuy MC, Duboeuf F, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin correlates with hip bone mineral density in elderly women. *J Bone Miner Res* **9**: 1591-1595, 1994.
- Kanai T, Takagi T, Masuhiro K, Nakamura M, Iwata M, Saji F. Serum vitamin K level and bone mineral density in postmenopausal women. *Int J Gynaecol Obstet* **56**: 25-30, 1997.
- Hirao M, Hashimoto J, Ando W, Ono T, Yoshikawa H. Response of serum carboxylated and undercarboxylated osteocalcin to alendronate monotherapy and combined therapy with vitamin K₂ in postmenopausal women. *J Bone Miner Metab* **26**: 260-264, 2008.
- Ishida Y, Kawai S. Comparative efficacy of hormone replacement therapy, etidronate, calcitonin, alfacalcidol, and vitamin K in postmenopausal women with osteoporosis. The Yamaguchi Osteoporosis Prevention Study. *Am J Med* **117**: 549-555, 2004.
- Shiraki M, Shiraki Y, Aoki C, Miura M. Vitamin K₂ (menatetrenone) effectively prevents fractures and sustains lumbar bone mineral density in osteoporosis. *J Bone Miner Res* **15**: 515-521, 2000.
- Ushiroyama T, Ikeda A, Ueki M. Effect of continuous combined therapy with vitamin K₂ and vitamin D₃ on bone mineral density and coagulofibrinolysis function in postmenopausal women. *Maturitas* **41**: 211-221, 2002.
- Knapen MH, Schurgers LJ, Vermeer C. Vitamin K₂ supplementation improves hip bone geometry and bone strength indices in postmenopausal women. *Osteoporos Int* **18**: 963-972, 2007.
- Ozuru R, Sugimoto T, Yamaguchi T, Chihara K. Time-dependent effects of vitamin K₂ (menatetrenone) on bone metabolism in postmenopausal women. *Endocr J* **49**: 363-370, 2002.
- Kaneko K, Kusunoki N, Hasunuma T, Kawai S. Changes of serum soluble receptor activator for nuclear factor-κB ligand after glucocorticoid therapy reflect regulation of its expression by osteoblasts. *J Clin Endocrinol Metab* **97**: E1909-E1917, 2012.
- Dovio A, Perazzolo L, Osella G, et al. Immediate fall of bone formation and transient increase of bone resorption in the course of high-dose, short-term glucocorticoid therapy in young patients with multiple sclerosis. *J Clin Endocrinol Metab* **89**: 4923-4928, 2004.
- Kuroki Y, Kaji H, Kawano S, et al. Short-term effects of glucocorticoid therapy on biochemical markers of bone metabolism in Japanese patients: a prospective study. *J Bone Miner Metab* **26**: 271-278, 2008.
- Kaji H, Kuroki Y, Murakawa Y, et al. Effect of alendronate on bone metabolic indices and bone mineral density in patients treated with high-dose glucocorticoid: a prospective study. *Osteoporos Int* **21**: 1565-1571, 2010.
- Kauh E, Mixson L, Malice MP, et al. Prednisone affects inflammation, glucose tolerance, and bone turnover within hours of treatment in healthy individuals. *Eur J Endocrinol* **166**: 459-467, 2010.
- Price PA. Vitamin K-dependent formation of bone Gla protein (osteocalcin) and its function. *Vitam Horm* **42**: 65-108, 1985.
- Koshihara Y, Hoshi K, Okawara R, Ishibashi H. Vitamin K stimulates osteoblastogenesis and inhibits osteoclastogenesis in human bone marrow cell culture. *J Endocrinol* **176**: 339-348, 2003.
- Takeuchi Y, Suzawa M, Fukumoto S, Fujita T. Vitamin K₂ inhibits adipogenesis, osteoclastogenesis, and ODF/RANK ligand expression in murine bone marrow cell cultures. *Bone* **27**: 769-776, 2000.
- Iwamoto J, Takeda T, Ichimura S. Effect of menatetrenone on bone mineral density and incidence of vertebral fractures in postmenopausal women with osteoporosis: a comparison with the effect of etidronate. *J Orthop Sci* **6**: 487-492, 2001.
- Yonemura K, Kimura M, Miyaji T, Hishida A. Short-term effect of vitamin K administration on prednisolone-induced loss of bone mineral density in patients with chronic glomerulonephritis. *Calcif Tissue Int* **66**: 123-128, 2000.
- Sasaki N, Kusano E, Takahashi H, et al. Vitamin K₂ inhibits glucocorticoid-induced bone loss partly by preventing the reduction of osteoprotegerin (OPG). *J Bone Miner Metab* **23**: 41-47, 2005.
- Ichikawa T, Horie-Inoue K, Ikeda K, Blumberg B, Inoue S. Steroid and xenobiotic receptor SXR mediates vitamin K₂-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. *J Biol Chem* **281**: 16927-16934, 2006.
- Walton RJ, Preston CJ, Russell RCG, Kenis JA. An estimate of

the turnover rate of rate of bone-derived olasm alkaline phosphatase in Paget's disease. Clin Chim Acta **63**: 227-229, 1975.
30. Peterkofsky B, Gosiewska A, Singh K, Pearlman S, Mahmoodian

F. Species differences in cis-elements of the pro α 1(1) procollagen promoter and their binding proteins. J Cell Biochem **73**: 408-422, 1999.

© 2016 The Japanese Society of Internal Medicine
<http://www.naika.or.jp/imonline/index.html>